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(71) Applicant: **Bristol-Myers Squibb Company**  
**345 Park Avenue**  
**New York, N.Y. 10154(US)**

(72) Inventor: **Lim, Gary M.F.**  
**104 Pine Ridge Road**  
**Fayetteville, New York 13066(US)**  
Inventor: **Roubie, John M.**  
**7205 Coventry Road North**  
**East Syracuse, New York 13057(US)**  
Inventor: **Garofalo, Elizabeth Ann**  
**7 Rustlers Road**  
**Marcellus, New York 13108(US)**

(74) Representative: **Kinzebach, Werner, Dr. et al**  
**Patentanwälte Reitstötter, Kinzebach und**  
**Partner Sternwartstrasse 4 Postfach 86 06 49**  
**W-8000 München 86 (DE)**

(54) **Process for the preparation of a cephalosporin antibiotic.**

(57) The present invention provides an anhydrous acylation process for the preparation of antibiotic, cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the  $\Delta^2$  isomer comprising the N-acylation of a silylated derivative of 7-amino-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate with the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride.

**EP 0 531 981 A1**

## BACKGROUND OF THE INVENTION

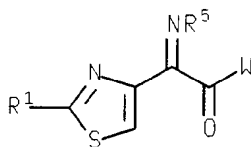
## 1. Field of the Invention

The present invention provides a chemical acylation process and, in particular, an anhydrous acylation process for the preparation of the antibiotic, cefepime dihydrochloride hydrate which is also known as 7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate. The present invention also provides a stable, crystalline salt of the synisomer of a thiazolyl intermediate and a process for the preparation thereof which may be used in the preparation of useful broad spectrum antibacterial agents.

## 2. Background Art

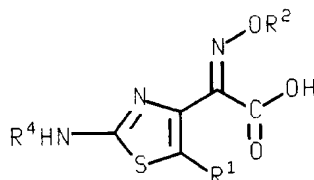
A large number of cephalosporin antibiotics are known which contain a 2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino acetic acid side chain which is coupled to the 7-amino group of a cephalosporanic acid by well-known acylation procedures. In most instances, it is necessary to protect the amino moiety and activate the carboxylic acid of the side chain as part of the acylation procedure. Consequently, the art discloses a large number of amino-protecting groups for the 2-amino group of the thiazole ring and a large number of activating groups for the carboxylic acid. The search for newer protecting groups and activating groups to produce the desired antibiotic is still the subject of numerous publications in view of the costs and toxicities associated with certain activating groups. Therefore, there is still a need in the preparation of useful broad spectrum antibiotics for a simple, stable, crystalline, economical, and non-toxic side chain having the desired geometric (z)-isomer which can be readily coupled to the 7-amino group of the cephalosporin nucleus. The following is representative of some of the thiazole side chain art.

In U.S. Patent No. 4,203,899 to Ochiai, et al., issued May 20, 1980, there is disclosed compounds of the Formula



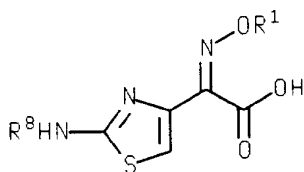
wherein R¹ represents amino, protected amino, hydroxyl, or protected hydroxyl; R⁵ represents hydroxyl or protected hydroxyl; and W represents hydroxyl, C₁-₄ alkoxy, halogen, or OM wherein M is an alkali metal.

U.K. Patent Application GB-2,144,424, published March 6, 1985, discloses the preparation of a series of pyridinium cephalosporin derivatives by various methods, including the use of the compound of Formula



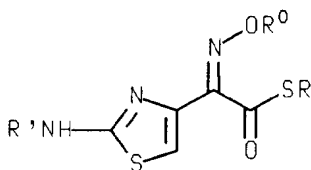
or a salt thereof wherein R¹ denotes a hydrogen or halogen atom; R² denotes a hydrogen atom or a C₁-₆ alkyl radical, and R⁴ represents hydrogen or an amino protective group, or with an activated derivative of this compound.

European Patent Application EP-160,546, published November 6, 1985, also discloses the preparation of a series of cephalosporin compounds by various methods, including the use of the substituted oxyiminothiazolyl acetic acid compounds of Formula



or a reactive derivative thereof, wherein  $R^8$  represents a hydrogen atom or a protective group for an amino group. Suitable examples of such reactive derivatives which are disclosed are mixed acid anhydrides, acid anhydrides, acid halides, active esters, active amides, and acid azides.

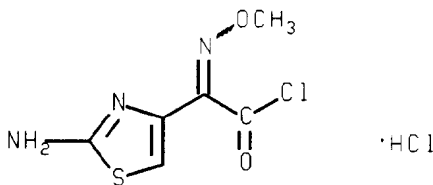
In U.S. Patent No. 4,385,181 to Farge, et al., issued May 24, 1983, there is disclosed thioesters of the Formula



wherein  $R^1$  represents hydrogen or a protecting radical,  $R^0$  represents hydrogen, alkyl, vinyl, cyanomethyl, or a protecting radical, and  $R$  represents alkyl, L-2-amino-2-carboxyethyl, phenyl, or a large number of various heterocyclic radicals listed in columns 4 to 8, as well as their syn- and anti-isomers and mixtures thereof.

In addition to the above-cited references, there exists a large number of references which disclose various protecting groups for the 2-amino substituent and an even larger number of activating groups/leaving groups of the carboxylic acid moiety which may be used in the acylation of a 7-amino cephalosporin compound.

However, the most relevant art is Czechoslovak Patent No. 238,950, published March 16, 1987, [Chemical Abstracts, Vol. 110, p. 544 (1989)] which discloses the compound of the instant invention having the Formula



wherein the compound is alleged to have the syn configuration. The only evidence presented for the product in the patent is a chlorine content of 99% to 100.5% of the theoretical value.

In connection with work on the development of new synthetic methods for the production of antibiotics, the present inventors, as well as others in the art, have felt the need for simple, convenient, economical, crystalline, stable, and non-toxic starting materials for use in the manufacture of antibiotics. Initial attempts to prepare and use the acid chloride of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid without the use of protecting groups were unsuccessful. However, the present inventors have now found that the compound of the instant invention can be prepared under specifically defined reaction conditions. This finding was further confirmed by the present inventors when they could not reproduce the teachings of the above-cited Czechoslovak patent. The desired syn-isomer of the acid chloride hydrochloride, which is necessary to produce the desired antibiotic, could not be repeated. Furthermore, additional experiments confirmed that the teachings of the art did not produce the hydrochloride salt of the desired syn-isomer of the acid chloride substantially free of the anti-isomer and having a proton nuclear magnetic resonance spectrum ( $^1H$  NMR) as described herein.

The broad spectrum antibiotic cefepime is disclosed by Aburaki, et al, in U.S. Patent No. 4,406,899, issued September 27, 1983 and its preparation is described by two Reaction Schemes in which the reactants and products required the use of blocking and deblocking groups. In the reaction scheme actually exemplified, the product required a chromatographic purification step to separate the mixture of  $\Delta^2$  and  $\Delta^3$  isomers and the cefepime product thereby produced is in the zwitterion form. However, the zwitterion form of cefepime is unstable at room and elevated temperatures.

Murray A. Kaplan et al, in U.S. Patent No. 4,910,301, issued March 20, 1990, discloses temperature stable crystalline salts of cefepime in dry powder form having excellent room and superior elevated temperature stability compared to the zwitterion form of cefepime described by Aburaki et al, U.S. Patent No. 4,406,899.

In U.S. Patent No. 4,868,294, issued September 19, 1989, Brundidge et al, describe the preparation of 7-amino-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate salts substantially free of the  $\Delta^2$  isomer and their use in an aqueous acylation procedure to prepare the antibiotic cefepime as the sulfate salt.

In U.S. Patent No. 4,754,031, issued June 28, 1988, Angerbauer et al, describe a process for the preparation of several cephalosporin antibiotics including cefepime in the zwitterion form. Although this process does not use protecting groups, it does use an anhydride for activation in an aqueous acylation reaction which requires chromatographic purification steps to give the zwitterion form of cefepime.

In U.S. Patent No. 4,943,631, issued July 24, 1990, Brian E. Looker describes an improved process for the preparation of the antibiotic cefepime as a hydroiodide salt. The process controls the formation of the undesirable  $\Delta^2$  isomer by employing a cephalosporin sulfoxide intermediate. However, the process described in the patent remains costly and inefficient since it introduces two additional steps to the prior art process and continues to use protecting groups which require blocking and deblocking procedures. Furthermore, the process requires the use of column chromatography as a purification method which is impractical on a manufacturing scale.

The preparation of the crystalline sulfate salt and zwitterion of cefepime which are described in the art use essentially the same aqueous acylation process and use various blocking and deblocking groups and active esters. In all instances, the preferred crystalline cefepime dihydrochloride hydrate form must be prepared via the purified zwitterion form of cefepime. Thus, there is a need to develop a simple, direct and cost effective acylation procedure which will avoid reaction steps to add and remove protecting groups, stereochemical controlling steps and chromatography procedures and, more importantly, an acylation procedure which will produce the desired antibiotic cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the  $\Delta^2$  isomer.

## SUMMARY OF THE INVENTION

The present invention provides a chemical acylation process and, in particular, an anhydrous acylation process for the preparation of the antibiotic, cefepime dihydrochloride hydrate substantially free of the anti-isomer and the  $\Delta^2$  isomer. The present invention also provides a stable, crystalline syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer which is used in the acylation process for the preparation of the broad spectrum antibiotic, cefepime.

## DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the proton nuclear magnetic resonance spectrum of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride of Example 10 in acetic acid- $d_4$  (100 MHz).

FIG. 2 shows the proton nuclear magnetic resonance spectrum of the product of Example 12 in acetic acid- $d_4$  (100 MHz).

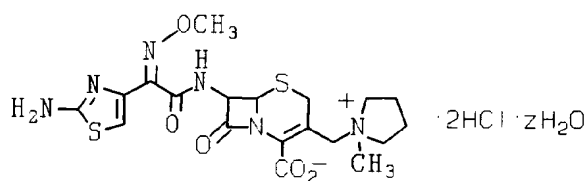
FIG. 3 shows the proton nuclear magnetic resonance spectrum of the product of Example 13 in acetic acid- $d_4$  (100 MHz).

FIG. 4 shows the proton nuclear magnetic resonance spectrum of the product of Example 14 in acetic acid- $d_4$  (100 MHz).

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an anhydrous acylation process for the N-acylation of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate with the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride which is substantially free of the anti-isomer to produce a temperature stable crystalline cefepime dihydrochloride hydrate substantially free of the anti-isomer and the

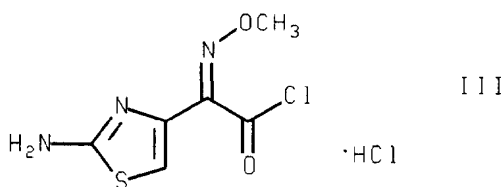
$\Delta^2$  isomer and is represented by Formula V wherein z is 1 or 2.



V

The advantages of the present anhydrous acylation process become apparent and may be appreciated by those skilled in the art when all the advantages are combined and considered as a whole. The elimination of formal amino and carboxyl protecting groups and the corresponding elimination of the additional chemical steps required for blocking and deblocking offer a distinct advantage in overall process efficiency and cost of materials over the previously known art processes. The present process additionally provides and maintains control of the stereochemical configuration of methoxyimino isomer and the  $\Delta^3$  double bond of the cephalosporin nucleus without the need to separate undesirable cephalosporin by-products by chromatography and without the need to use stereochemical controlling sulfoxide intermediates such as described in U.S. Patent No. 4,043,631. Another advantage of the present invention is the preparation and use of the unprotected crystalline hydrochloride salt of syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride of Formula III which avoids unusual and sometimes complex organic leaving groups described in the art. The use of the simple chloride ion as a leaving group avoids the use of potential toxic leaving groups such as 2-mercaptobenzothiazole. A further advantage of the preferred embodiment of the present anhydrous acylation process is to provide the desired temperature stable crystalline cefepime dihydrochloride hydrate directly from the reaction mixture of the acylation process without the need to prepare and isolate the sulfate salt or zwitterion of cefepime. The present process also provides the water soluble crystalline cefepime dihydrochloride monohydrate or dihydrate substantially free of the anti-isomer and the  $\Delta^2$  isomer in high yield directly from the aqueous phase of the diphasic solution.

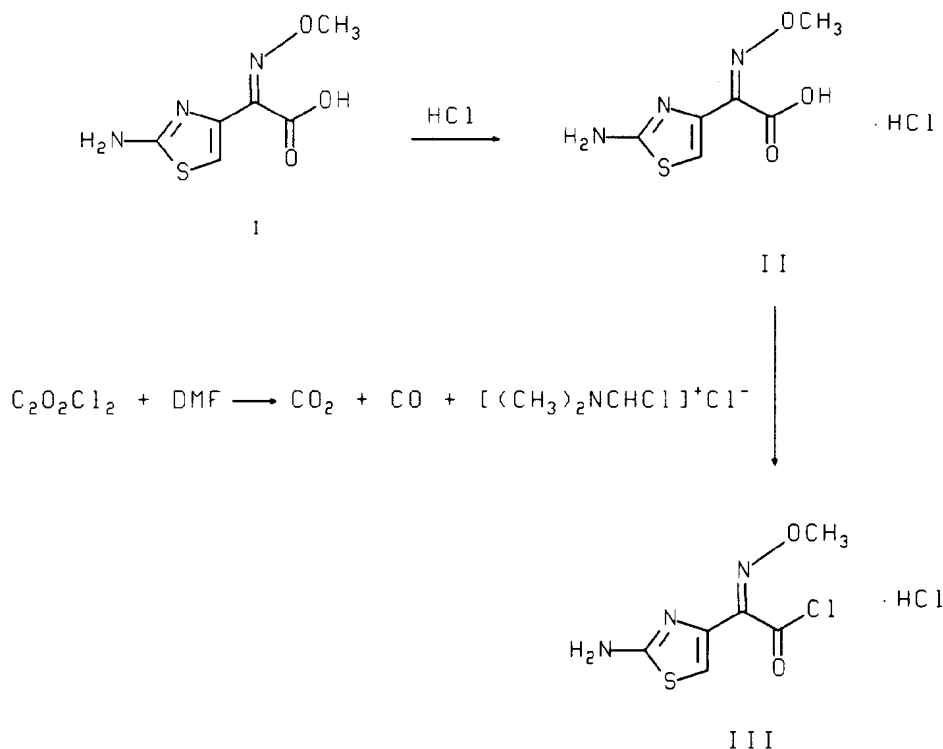
The present invention also provides a stable, crystalline syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride which is substantially free of the anti-isomer and is represented by Formula III.



As a result of being substantially free of the anti-isomer, compound III is convertible to broad spectrum cephalosporins which themselves are substantially free of the anti-isomer, without the need for chromatographic separation of the syn- and anti-isomers. As a result of the enhanced stability, compound III may be isolated and stored and, when desired, compound III may be converted to end products in a different solvent which is advantageous for the production of the desired antibiotic substantially free of the  $\Delta^2$  isomer. An additional advantage of the intermediate of Formula III is that it does not require blocking (protection) of the amino group prior to acylation or deblocking (deprotection) of the amino group after acylation, thus, offering process efficiency. A further advantage of the acid chloride of Formula III is its use in the acylation process to produce broad spectrum cephalosporins. In contrast to other methods such as described in Aburaki, et al., U.S. Patent No. 4,406,899, the intermediate of Formula III has a chloride ion as a simple and non-toxic leaving group with no precaution necessary to remove it from the desired antibiotic as is the instance with most other leaving groups known in the art. Also, some of the intermediates known in the art which contain other leaving groups are difficult to prepare while other intermediates which contain leaving

groups such as 2-mercaptobenzothiazole have been found to be toxic [Chem. Abstracts, 1989, Vol. 111 (3), 19243p].

### Reaction Scheme 1



The syn-isomer of the acid chloride hydrochloride of Formula III may be prepared from the syn-isomer of the acid of Formula I as outlined in Reaction Scheme 1. The acid of Formula I is first converted to the corresponding hydrochloride salt of Formula II by methods known in the art and then, if desired, isolated as an anhydrous, crystalline compound of Formula II. The formation of the hydrochloride salt is advantageously carried out with at least one molar equivalent of gaseous hydrogen chloride in an inert organic solvent such as toluene, acetonitrile, dichloromethane, acetone, benzene, xylene, cyclohexane, hexanes, dioxane or diethyl ether at a temperature of about -10°C to about 50°C. Preferably, the reaction is carried out in toluene, dichloromethane or acetonitrile and the hydrochloride of Formula II thereby produced may be isolated or used in situ. When the reaction is conducted in acetonitrile, the resulting hydrochloride of Formula II tends to retain loosely bound solvent. Consequently, it is advantageous to use the acid hydrochloride of Formula II from acetonitrile in a reasonable period of time in the next step to avoid solvate displacement by atmospheric moisture. Most preferably, the reaction is carried out in toluene or dichloromethane at about 0°C to room temperature.

The acid salt of Formula II is then advantageously treated with a chlorinating agent and, most preferably, with oxalyl chloride in combination with dimethylformamide to produce the stable, crystalline syn-isomer of compound III. As demonstrated herein, the use of other known chlorinating agents can result in isomerization to give the undesirable anti-isomer or mixtures of syn- and anti-isomers. Additionally, chlorinating agents, such as phosphorous pentachloride, may result in the chlorination of the 5-position on the thiazole ring which would then result in an undesirable impurity in the antibiotic. The present inventors have discovered that, in addition to the preparation of the acid hydrochloride of Formula II, the appropriate selection of chlorinating agent and reaction conditions, such as solvent and temperature, are critical in the process for the preparation of the syn-isomer of compound III which is substantially free of the anti-isomer.

Chlorinating methods, which are generally used for activating acids, are well-known in the art. Phosphorous pentachloride, which is the most widely used chlorinating agent, is not suitable for the chlorination of compound II since it also results in the isomerization of the methoxyimino group to produce the undesirable anti-isomer of compound III. This is clearly demonstrated in Examples 12, 13, 14, and 16 as described herein. Another known chlorinating method is the use of oxalyl chloride in combination with dimethylformamide. However, the present inventors have discovered that the oxalyl chloride method, wherein dimethylformamide is used as a catalyst, does not produce substantial amounts of the desired syn-isomer of compound III. This is also clearly demonstrated in Example 15 as described herein. After extensive studies, the present inventors have discovered that the use of dimethylformamide in an amount less than an equimolar amount relative to the amount of oxalyl chloride is deleterious to the production of the desired syn-isomer of the acid chloride hydrochloride of Formula III. Most preferably, the molar amount of dimethylformamide should exceed the molar amount of oxalyl chloride. The inventors have also found that the use of excess molar amounts of dimethylformamide is also deleterious to both the reaction and to the stability of the desired product. Thus, the inventors have discovered a method to control the instability of the reaction materials to either excess chloride ion generated by oxalyl chloride or excess dimethylformamide which are critical for production of the stable, crystalline syn-isomer of compound III which is substantially free of the anti-isomer. In the instance, when the conversion of the compound of Formula II to the compound of Formula III is not complete, there will remain a small amount of the syn-isomer of the acid of Formula II in the isolated product of compound III. The presence of some unreacted compound II in the product of compound III and small amounts of the anti-isomer of compound III do not affect the subsequent acylation reaction for the successful production of the desired antibiotic which is substantially free of the anti-isomer of said antibiotic.

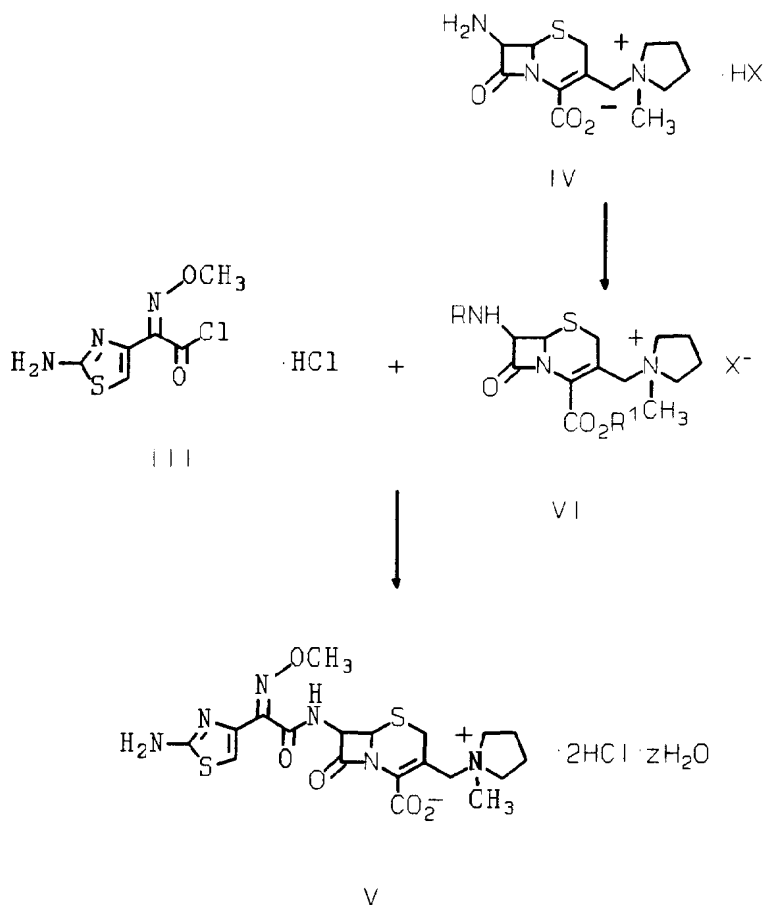
The present inventors have also found that the temperature and reaction solvent of the chlorinating reaction are also critical. Preferably, the reaction is conducted in an inert organic solvent, such as dichloromethane, chloroform or acetonitrile, at a temperature of less than  $-10^{\circ}\text{C}$ . Most preferably, the reaction is advantageously carried out in dichloromethane at about  $-15^{\circ}\text{C}$  to about  $-40^{\circ}\text{C}$ .

The use of the syn-isomer of the acid chloride hydrochloride of Formula III to prepare useful broad-spectrum antibiotics by way of a general acylation reaction is illustrated in Reaction Scheme 2. More specifically, Reaction Scheme 2 illustrates the use of the acid chloride of Formula III to prepare the broad spectrum antibiotic cefepime which is substantially free of the anti-isomer and the  $\Delta^2$  isomer. Furthermore, the acid chloride hydrochloride of Formula III may be used to prepare cephalosporin antibiotics having the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl attached to the 7-amino group of the cephalosporin nucleus such as cefodizime, cefmenoxime, cefotaxime, cefpirome, cefpodoxime, cefquinome, cefteteram, ceftiofur, cefetamet and cefuzonam.

Furthermore, to confirm that the acid chloride hydrochloride product of the prior art is in the anti-isomer and not in the desired syn-isomer, the present inventors substituted the product of the prior art such as that produced in Example 14 for the syn-isomer of the compound of Formula III in the acylation reactions illustrated in Reaction Scheme 2 and in Example 4. The resulting cephalosporin product which was produced as described in Examples 17, 18 and 23 was compared to the cefepime antibiotic produced by the present invention. As can be seen by the comparison in Example 19, the anti-cefepime produced by teachings of the prior art is not the same as the useful broad-spectrum syn-cefepime produced by use of the present invention.

As defined herein and in the claims, the term "substantially free" means that the compound contains less than about 5% of the undesirable isomer. Preferably, the compound contains less than about 1% of the undesirable isomer.

According to the process of the present invention, the broad-spectrum antibiotic cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the  $\Delta^2$  isomer is prepared by the N-acylation of a compound of Formula IV with the syn-isomer of the acid chloride hydrochloride of Formula III as illustrated in Reaction Scheme 2.

Reaction Scheme 2

The 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]-ceph-3-em-4-carboxylate salt which is substantially free of the  $\Delta^2$  isomer and represented by Formula IV wherein HX is HCl, HI or  $\text{H}_2\text{SO}_4$  may be prepared by the general procedures described by S.P. Brundidge, et al. in U.S. Patent No. 4,868,294.

The cephalosporin intermediate of Formula IV wherein HX is preferably HI may advantageously be silylated in an inert organic solvent to form an *in situ* solution of the soluble silylated derivative represented by Formula VI wherein R and R<sup>1</sup> each are independently H or a silyl group or a mixture thereof. In one <sup>1</sup>H NMR study it was determined that R<sup>1</sup> is mostly silylated and R is mostly hydrogen. It should be appreciated by those skilled in the art that, due to the constant change of equilibration of the different species present in the solution, it is difficult to identify the specific compound of Formula VI generated *in situ* in the reaction mixture at any particular time. However, it is important to add sufficient silylating agent and, if necessary, a base to solubilize the cephalosporin intermediate of Formula IV before the anhydrous solution is treated with the compound of Formula III. Silylating agents which may be used are well known to those skilled in the art and are, for example, trimethylchlorosilane, trimethyliodosilane, hexamethyldisilazane, tert-butyldimethylchlorosilane, trimethylsilylacetamide, bis-(trimethylsilyl)acetamide, bis-(trimethylsilyl)urea or the like. Preferably, trimethylchlorosilane or a mixture of trimethylchlorosilane and hexamethyldisilazane and, most preferably, a mixture of trimethylchlorosilane and hexamethyldisilazane may be used in the acylation process.

Although at least one molar equivalent of a silylating agent and at least one molar equivalent of a base are required in the reaction, it was found in practice that it was advantageous to use about two molar equivalents of a silylating agent and about two molar equivalents or less of a base to obtain a solution of the intermediate of Formula VI. However, when the silylating agent is a mixture of trimethylchlorosilane and hexamethyldisilazane, it is preferable not to add any base to the preparation of the soluble derivative of Formula VI. As will be appreciated by those skilled in the art, hexamethyldisilazane, as a silylating agent, will produce a base by-product which is sufficient to neutralize some of the acid which is produced. Since

excess base is deleterious to the production of the desired antibiotic which is substantially free of the  $\Delta^2$  isomer, a mixture of trimethylchlorosilane and hexamethyldisilazane is most preferred in the present invention. The amount of  $\Delta^3$  to  $\Delta^3$  isomerization in the cephalosporin nucleus is sensitive to the reaction conditions employed in the preparation of the soluble silylated derivative of Formula VI and in the N-acylation thereof with the acid chloride hydrochloride of Formula III to produce the compound of Formula V. The amount of isomerization is dependent on factors such as the amount and order of addition of base, the solvent and the temperature employed in the process. Most importantly, basic conditions resulting from the use of excess base or from the addition of base prior to the addition of silylating agent or acid chloride hydrochloride of Formula III will increase isomerization of the cephem double bond from  $\Delta^3$  to  $\Delta^2$ . Therefore, it is most preferred to maintain non-basic reaction conditions during the anhydrous acylation process.

Suitable bases which may be used in the process are inorganic and organic bases which are suitable acid acceptors such as  $\text{NaHCO}_3$ ,  $\text{KHCO}_3$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{K}_2\text{CO}_3$ , ammonia, primary amine, secondary amine, tertiary amine or the like. Most preferably, organic bases which may be used in the process are, for example, 1,8-diazabicyclo-[5.4.0]undec-7-ene, N-methylmorpholine, 2,6-lutidine, 2-methyl-6-ethylpyridine, N,N-dimethylaniline, N,N-diethylaniline, triethylamine, diisopropylethylamine or the like. Preferably, and if required, N-methylmorpholine or triethylamine or a mixture thereof may advantageously be used in the silylation and N-acylation reactions. Most preferably, N-methylmorpholine or triethylamine is employed in the N-acylation of the compound of Formula VI with the compound of Formula III.

The soluble silylated derivative of Formula VI which is generated *in situ* is then treated with the acid chloride hydrochloride of Formula III and, preferably, with one molar equivalent and, most preferably, a slight excess of the acid chloride hydrochloride of Formula III followed by an equimolar amount and, preferably, less than an equimolar amount of a base relative to the amount of the compound of Formula III to produce a mixture containing the desired antibiotic. Preferably, the amount of acid chloride hydrochloride of Formula III and the base is slowly added in portions. Although the addition of reactants may be carried out all at once if the temperature and non-basic conditions of the reaction can be controlled, it is advantageous to add the reactants in two or three portions to ensure complete reaction.

Suitable solvents which may be used in the process are all inert organic solvents in which the silylated derivative of Formula VI is soluble and in which isomerization of the  $\Delta^3$  double bond is minimized, for example, toluene, tetrahydrofuran, acetone, acetonitrile, dichloromethane, chloroform, dimethylacetamide or the like, or mixtures thereof. Most preferably, acetonitrile or dichloromethane may be used in the process. The process of the present invention may be carried out at a temperature of about  $-60^\circ\text{C}$  to about  $+50^\circ\text{C}$  and, preferably, at about  $-40^\circ\text{C}$  to about room temperature. The preparation of the silylated compound of Formula VI is advantageously carried out at about  $-10^\circ\text{C}$  to about room temperature while the N-acylation is advantageously carried out at about  $-40^\circ\text{C}$  to about  $0^\circ\text{C}$ .

When the N-acylation of the soluble silylated compound of Formula VI is complete as ascertained by known detection methods available in the art, for example, thin-layer chromatography, high pressure liquid chromatography and spectroscopic methods; then, according to the preferred process of the present invention, sufficient water is added to the reaction mixture to dissolve, if desired, visible solids and produce a biphasic solution of organic phase and water. The amount of water to be added to the reaction mixture is determined by the selection and quantity of the inert organic solvent used in the process and should be sufficient to give or cause phase separation. Once phase separation has occurred, it is advantageous to separate and discard the organic phase to produce an aqueous rich solution containing the desired antibiotic. The aqueous rich solution is then treated with a sufficient amount of an acid or soluble non-toxic salt thereof such as hydrochloric acid, sodium chloride, ammonium chloride, potassium chloride, sulfuric acid, sodium sulfate, potassium sulfate, ammonium sulfate, phosphoric acid, sodium phosphate, potassium phosphate, ammonium phosphate, nitric acid, sodium nitrate, potassium nitrate or the like to provide a sufficient amount of the desired counter anion to ensure crystallization of the desired cefepime salt and, optionally, diluted with an appropriate water-miscible organic solvent such as methyl ethyl ketone, acetone, isopropanol, butanol or the like to induce or complete crystallization. Preferably, the aqueous rich solution is treated with a sufficient amount of sulfuric acid to crystallize the sulfate salt of cefepime which is substantially free of the anti-isomer and the  $\Delta^2$  isomer. The cefepime sulfate may then be converted to the preferred crystalline cefepime dihydrochloride monohydrate by the method described by Kaplan et al., in U.S. Patent No. 4,910,301. The cefepime sulfate which is produced in the present process may be neutralized with a base and, preferably, with a weakly basic ion-exchange resin known in the art and, preferably, commercially available such as Amberlite LA-2, Dowex WGR, Bio-Rad AG3-X4A, Amberlite IRA 93, Amberlite IRA 35 or the like to produce an aqueous or aqueous-organic solution containing the zwitterion form of cefepime. The solution is then treated with a sufficient amount of hydrochloric acid and,

optionally, with a water-miscible organic solvent to induce the crystallization of the preferred crystalline cefepime dihydrochloride hydrate. Most preferably, the aqueous rich solution obtained from the anhydrous acylation of the present process is treated with a sufficient amount of hydrochloric acid to induce and ensure crystallization of said antibiotic, cefepime dihydrochloride hydrate when a water-miscible organic solvent such as acetone is added. The amount of water-miscible organic solvent to be added should be sufficient to produce complete crystallization of said antibiotic and, advantageously, in an amount of about 2 to about 9 volumes of the aqueous phase to produce the temperature stable crystalline cefepime dihydrochloride monohydrate or dihydrate which is substantially free of the anti-isomer and the  $\Delta^2$  isomer.

When it is desired to prepare only the cefepime dihydrochloride monohydrate, the rich aqueous solution from the anhydrous acylation is advantageously treated with a sufficient amount of hydrochloric acid and diluted with an appropriate amount of a water-miscible organic solvent as described herein to ensure the crystallization of the desired monohydrate form. Alternatively, when it is desired to prepare the stable cefepime dihydrochloride dihydrate, the rich aqueous solution is advantageously treated with a greater equivalent concentration of hydrochloric acid and an amount of water-miscible organic solvent to hold the crystallization at the cloud point before additional organic solvent is added to complete the crystallization. However, it should be appreciated by those skilled in the art that if the isolation step from the aqueous rich solution of the process is not carefully controlled, it is possible that a mixture of crystalline cefepime dihydrochloride monohydrate and dihydrate forms may be produced. In any event, the preparation of only one of the desired hydrates can be prepared from either hydrate or a mixture of hydrates by following the recrystallization procedures described herein.

The crystalline cefepime dihydrochloride monohydrate prepared by the present process may be used to prepare a stable crystalline cefepime dihydrochloride dihydrate by recrystallization under controlled concentrations of solvent and hydrochloric acid, and the amount of time held at the cloud point (initial crystallization) as described herein. Alternatively, the crystalline dihydrochloride dihydrate prepared by the present process may also be used to prepare a stable crystalline cefepime dihydrochloride monohydrate by recrystallization under different controlled conditions as described herein. Thus the process of the present invention may be used to produce either the desired monohydrate or dihydrate of said antibiotic.

In contrast to the labile cefepime dihydrochloride dihydrate described in U.S. Patent No. 4,910,301 which readily loses the second mole of water, the crystalline cefepime dihydrochloride dihydrate which may be produced by the present process has been found to have a well-defined crystalline structure which retains the second mole of water. The new crystalline dihydrate form (needle-like crystals) has been found to be remarkably stable and its crystal morphology does not change under various conditions, for example, in air at a temperature of 70 °C for more than two months, under vacuum with P<sub>2</sub>O<sub>5</sub> at 50 °C for 48 hours, under oven drying at 70 °C for 96 hours and under high or low relative humidity conditions. The crystalline dihydrate exhibits characteristic infrared absorption peaks at 3574 cm<sup>-1</sup> and 3432 cm<sup>-1</sup> as indicated by FT-IR diffuse reflectance spectroscopy with KBr and a 13 mm sample cup using a Nicolet 20SX spectrometer. This temperature and moisture stable crystalline dihydrate form of cefepime is also characterized by an X-ray powder diffraction pattern as shown in Table 1.

TABLE 1

Stable Cefepime Dihydrochloride Dihydrate	
d	I/I <sub>0</sub> (%)
13.14	15
12.78	13
8.82	24
6.62	18
6.41	100
4.94	17
4.79	10
4.74	12
4.52	13
4.41	36
4.1	63
3.75	50
3.6	11
3.53	16
3.41	36
3.31	9
3.19	22
2.84	30
2.67	16
2.62	6
2.57	14
2.5	4
2.48	9
2.27	15

In Table I the "d" refers to interplanar spacings and "I/I<sub>0</sub>" refers to relative percent intensities. The X-ray pattern was collected with a Rigaku Geigerflex X-Ray Diffractometer and a nickel filtered copper (K<sub>α</sub>) radiation wavelength of 1.5425 Å;

Thus, an embodiment of the present invention provides a process for the preparation of antibiotic, cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the Δ<sup>2</sup> isomer which comprises reacting a silylated derivative of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate with the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer in an inert organic solvent.

A preferred embodiment of the present invention further comprises the preparation of the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer by reacting the anhydrous acid hydrochloride salt of the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid with a mixture containing at least one molar equivalent of oxalyl chloride and at least one molar equivalent to a slight excess of dimethylformamide to an amount of said oxalyl chloride in an inert organic solvent at a temperature of less than -10 ° C.

Another preferred embodiment of the present invention further comprises the preparation of the silylated derivative by reacting 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate salt in an inert organic solvent with a silylating agent.

A more preferred embodiment of the present invention provides a process for the preparation of antibiotic, cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the Δ<sup>2</sup> isomer which comprises reacting a silylated derivative of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate with the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer in an inert organic solvent and further comprises the addition of a sufficient amount of water to the reaction mixture to produce an organic-aqueous diphasic solution; and then adding a sufficient amount of an acid or soluble non-toxic salt thereof and, optionally, a water-miscible organic solvent to the separated aqueous solution.

The most preferred embodiment of the present invention provides a process for the preparation of the antibiotics, cefepime dihydrochloride monohydrate and cefepime dihydrochloride dihydrate directly from the

rich aqueous solution of the present anhydrous acylation process.

The utility of cefepime (Compound V) is shown in Abruaki et al, U.S. Patent No. 4,406,899. The stable dihydrate form of cefepime produced by the present process exhibits the antibiotic properties of the above-mentioned cefepime of U.S. Patent No. 4,406,899 and finds utility as an antibiotic in like manner.

It is understood that the specification and the examples are illustrative and are not to be construed as limiting the scope of the invention.

#### DESCRIPTION OF SPECIFIC EMBODIMENTS

##### Example 1

##### Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt

A suspension of (25 g, 124.25 mmol) 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid in toluene (250 ml) was gassed with HCl at 20°-28° C. The HCl was introduced subsurface in two aliquots of 8.1 g (222.2 mmol) and 4.8 g (131.7 mmol) with 30 minutes agitation between aliquots. After 1 hour at 20° C, the product was collected by filtration under a nitrogen atmosphere, washed with toluene (50 ml) and hexane (250 ml), and dried at 20°-25° C in vacuo to yield 28.68 g (97%) of the title compound.

##### Example 2

##### Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

To a solution of (0.77 ml, 10 mmol) dimethylformamide in dichloromethane (40 ml) at 5° C was added (0.89 ml, 10 mmol) 98% oxalyl chloride in dichloromethane (4.1 ml). Dropwise addition kept the temperature at 4°-5° C. To the resulting suspension, cooled to -27° C, was added (2.37 g, 10 mmol) 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride prepared in Example 1. The suspension was stirred for 2.5 hours at -25° C. Filtration under a nitrogen atmosphere and washing with dichloromethane (50 ml) and hexane (100 ml) gave 1.78 g (69.5%) of white, crystalline title compound after drying at 20° C in vacuo.

The title acid chloride acylated 7-amino desacetoxy cephalosporanic acid diphenylmethyl ester hydrochloride in pyridine solution to give a single zone (TLC) product coincident with and inseparable from an authentic sample of the desired desacetoxy cephalosporin ester.

##### Example 3

##### Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

To a solution of (1.55 ml, 20 mmol) dimethylformamide in dichloromethane (80 ml) at 5° C was added (1.78 ml, 20 mmol) of 98% pure oxalyl chloride in dichloromethane (8.2 ml). Addition time was 5 minutes at 5°-8° C. The resulting suspension was agitated 10 minutes at 5° C and then cooled to -30° C. 2-(2-Aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride (4.75 g, 20 mmol) from Example 1 was added. The suspension was stirred for 2.5 hours at -25° to -30° C. Filtration under a nitrogen atmosphere and washing with dichloromethane (75 ml) and hexane (100 ml) gave 3.57 g (69.7%) of crystalline title compound after drying at 20° C in vacuo.

An aliquot of the solid acid chloride hydrochloride product acylated 7-amino desacetoxy cephalosporanic acid diphenylmethyl ester hydrochloride in pyridine solution to give essentially single zone (TLC) product which was coincident with and inseparable from an authentic sample of the desired desacetoxy cephalosporin ester.

## Example 4

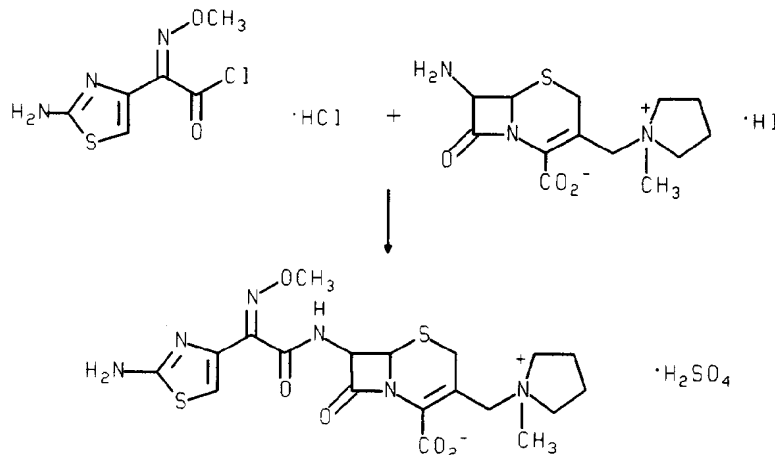
Preparation of 7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate (cefepime)

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25 7-Amino-3-[(1-methyl-1-pyrrolidinio)methyl] ceph-3-em-4-carboxylate monohydroiodide (0.85 g, 2.0 mmol) [prepared according to the procedures described by S. P. Brundidge, et al., in U.S. Patent No. 4,714,760] was dissolved in 9 ml of acetone-water (2:1) with triethylamine at pH 6.5 and 20 °C. Syn 2-(2-amino-thiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (0.56 g, 2.2 mmol) [prepared in Example 3] was added using triethylamine to control the pH in the 5-7 range. Assay of the resulting solution by high pressure liquid chromatography showed 58% yield of the desired cephalosporin (cefepime). Acidification with sulfuric acid to pH 2.2 gave 0.63 g of the title antibiotic as its sulfate salt (51% activity yield), as described by Aburaki, et al., in U.S. Patent No. 4,406,899, issued September 27, 1983, and by Kaplan, et al., in U.S. Patent No. 4,910,301, issued March 20, 1990.

## 35 Example 5

Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

40 To a solution of (9.75 ml, 125.9 mmol) dimethylformamide in dichloromethane (450 ml) at 5 °C was added dropwise a solution of (11.21 ml, 125.9 mmol) oxalyl chloride (98%) in dichloromethane (15 ml). Addition was completed in 10 minutes at 5 °-7 °C. To the resulting slurry, cooled to -25 °C, was added (28.5 g, 119.9 mmol) syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt in one aliquot. The slurry was stirred for 3.5 hours at -25 ° to -30 °C, filtered under a nitrogen atmosphere, washed with dichloromethane (100 ml) and hexane (400 ml), and dried at 20 °-25 °C in vacuo. Yield of crystalline title compound was 30.7 g (72.5%).

45 The title acid chloride acylated 7-amino desacetoxyccephalosporanic acid diphenylmethyl ester hydrochloride in pyridine solution to give essentially one zone (TLC) of the desired desacetoxyccephalosporin ester by reference to an authentic sample.

50 The title acid chloride (200 mg, 0.8 mmol) was hydrolyzed in water. <sup>1</sup>H NMR of the isolated product was identical to the starting syn acid.

## Example 6

Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

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To a solution of (8.13 ml, 105 mmol) dimethylformamide in dichloromethane (350 ml) at 5 °C was added dropwise (9.34 ml, 105 mmol) oxalyl chloride (98% pure) in dichloromethane (5 ml). Maximum temperature reached during the addition was 7 °C. The resulting suspension was stirred for 10 minutes at 5 °C and then

cooled to -27 °C. 2-(2-Aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride (23.8 g, 100 mmol) was added in one aliquot. The suspension was stirred for 2.5 hours at -25 ° to -30 °C, filtered under a nitrogen atmosphere, washed with dichloromethane (25 ml) and hexane (125 ml), and dried at 20 °C in vacuo. Yield was 21.39 g (83.5%) of crystalline acid chloride hydrochloride.

Anal. Calcd. for C <sub>6</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub> SCl <sub>2</sub> :				
	C, 28.14;	H, 2.76;	N, 16.41;	S, 12.52;
Found:	C, 28.25;	H, 2.93;	N, 16.32;	S, 12.67.

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 3.93 (CH<sub>3</sub>), 7.04 (H5).

#### Example 7

##### Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt

A suspension of (87 g, 432.4 mmol) syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid in toluene (870 ml) at 22 °C was gassed with two aliquots of HCl; 17.5 g, 480 mmol in 30 minutes and 15.0 g, 410 mmol in 20 minutes with 20 minutes agitation between aliquots. The slurry was agitated 1.5 hours at 25 °C, filtered under a nitrogen atmosphere, washed with toluene (100 ml) and hexane (400 ml), and dried at 20 °-25 °C in vacuo. Yield of the title compound was 100.2 g (97.5%).

Anal. Calcd. for C <sub>6</sub> H <sub>8</sub> N <sub>3</sub> O <sub>3</sub> SCl:				
	C, 30.32;	H, 3.39;	N, 17.68;	S, 13.49;
	Cl, 14.92;			
Found:	C, 30.51;	H, 3.39;	N, 17.54;	S, 13.37;
	Cl, 14.90.			

#### Example 8

##### Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

To a solution of (32.4 ml, 419.7 mmol) dimethylformamide in dichloromethane (400 ml) at 5 °C was added (37.4 ml, 419.7 mmol) 98% oxalyl chloride dropwise. The resulting suspension was cooled to -25 °C and added to a -25 °C suspension of (95 g, 399.7 mmol) syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride from Example 7. The suspension was stirred for 2.5 hours at -25 ° to -28 °C, filtered under a nitrogen atmosphere, washed with dichloromethane (100 ml) and hexane (500 ml), and dried at 20 °-25 °C in vacuo. The yield of crystalline title compound was 84.3 g (82.3%).

Anal. Calcd. for C <sub>6</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub> SCl <sub>2</sub> :				
	C, 28.14;	H, 2.76;	N, 16.41;	S, 12.52;
Found:	C, 27.90;	H, 3.10;	N, 16.14;	S, 12.27.

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 3.95 (CH<sub>3</sub>), 7.04 (H5).

#### Example 9

##### Preparation of 7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-methyl]-ceph-3-em-4-carboxylate (cefepime)

To a solution of 240 ml acetone and 80 ml water was added 20.0 g of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]-ceph-3-em-4-carboxylate hydroiodide (0.047 mol) and set stirring. Using a Radiometer ABU80 auto titrator with end point set at pH 6.5 and filled with N-methylmorpholine, syn 2-(2-aminothiazol-

4-yl)-2-methoxyimino acetyl chloride hydrochloride (20.0 g, 0.0785 mol) [prepared in Example 5] was added in 4 portions at 5 minute intervals keeping the pH at 6.5. After the addition was completed, the thin slurry was stirred for an additional 20 minutes at room temperature. The pH of the reaction mixture was lowered to pH 2.65 with 21 ml of 6N H<sub>2</sub>SO<sub>4</sub>. Precipitation of the title compound occurred. The slurry was seeded and stirred at room temperature for 20 minutes. The pH of the slurry was readjusted to 1.8 with 16 ml of 6N H<sub>2</sub>SO<sub>4</sub>, and stirring was continued for another 60 minutes. The slurry was filtered in vacuo and washed with 70 ml water-acetone (1:1) and then 70 ml acetone to give 24.09 g (88.5% stoich. weight yield) of the title compound, which is identical to the compound of Example 4 and to the cefepime described by Aburaki, et al., in U.S. Patent No. 4,406,899, issued September 27, 1983, and by Kaplan, et al, in U.S. Patent No. 4,910,301, issued March 20, 1990.

#### Example 10

##### Preparation of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

To a solution of dimethylformamide (8.76 ml, 0.113 mole) in dichloromethane (375 ml) at 5° C, add oxalyl chloride (9.64 ml, 0.111 mole) dropwise keeping the temperature at 5°-6° C. Agitate the suspension for 10 minutes and then cool to -25° C. Add syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt (25.0 g) in aliquots over 11 minutes under a dry nitrogen atmosphere. Agitate the slurry for 2.5 hours at -25° C. Filter the product in a dry nitrogen atmosphere, and wash the cake with dichloromethane (80 ml). Dry the product at 20°-25° C in vacuo over P<sub>2</sub>O<sub>5</sub> to yield 23.88 g (88.6%) of the title compound as a pale-yellow, crystalline solid.

Anal. Calcd. for C <sub>6</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub> SCl <sub>2</sub> :				
	C, 28.14; Cl, 27.68;	H, 2.76;	N, 16.41;	S, 12.52;
Found:	C, 28.06; Cl, 27.23.	H, 2.71;	N, 16.26;	S, 12.30;

The product of the foregoing experiment was characterized by proton nuclear magnetic resonance spectrum in acetic acid-d<sub>4</sub> (<sup>1</sup>H NMR), as shown in Figure 1.

<sup>1</sup>H NMR (CD<sub>3</sub>CO<sub>2</sub>D) δ: 4.14 (CH<sub>3</sub>), 7.10 (H5). Residual acid hydrochloride level from CH<sub>3</sub> (4.11) integral is 5.1%. A trace level of isomeric H5 is seen at 7.67 ppm.

#### Example 11

##### Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

To a solution of dimethylformamide (17.92 ml, 231.9 mmol) in dichloromethane (375 ml) at 5° C was added oxalyl chloride (19.76 ml, 220.8 mmol). Addition time was 15 minutes at 5°-6° C. The resulting suspension was agitated 10 minutes at 5°-6° C and then cooled to -25° C. 2-(2-Aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride (25.0 g, 105.2 mmol) was added. The resulting solution was seeded with the title compound to produce the product slurry. The suspension was agitated for 3.5 hours at -25° C, filtered in a dry nitrogen atmosphere, washed with dichloromethane (150 ml), and dried at 20°-25° C in vacuo. Yield was 9.61 g (35.7%) of crystalline title compound.

An aliquot of the solid acid chloride hydrochloride product acylated 7-amino desacetoxy cephalosporanic acid diphenylmethyl ester hydrochloride in pyridine solution to give essentially single zone (TLC) product which was coincident with and inseparable from an authentic sample of the desired desacetoxy cephalosporin ester.

#### Example 12

##### Preparation of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

The experimental procedure of Example 1 in Czechoslovakian Patent No. 238,950 was repeated as follows:

A sample of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (4.0 g) having a KF of 0.06% was slurried in 30 ml of benzene at 21 °C. One drop of dimethylformamide was added, followed by 5.0 grams of powdered phosphorous pentachloride in one aliquot. The temperature rose to 34 °C in approximately 2 minutes and was then raised to 40 °C in 1 minute to provide a complete solution. The solution was allowed to cool, and at 36 °C, a precipitate formed. After stirring for 30 minutes, the temperature was 22 °C. The light-yellow solid was collected by filtration under a dry nitrogen atmosphere and washed with 30 ml of benzene and 20 ml of heptane. Yield was 2.88 g after drying in vacuo over P<sub>2</sub>O<sub>5</sub> at 20 °-25 °C for 18 hours.

The product of the foregoing experiment was characterized by proton nuclear magnetic resonance spectrum in acetic acid-d<sub>4</sub> (<sup>1</sup>H NMR) as shown in Figure 2 which shows the H5 at 7.56 ppm and CH<sub>3</sub> at 4.34 ppm. This spectrum is consistent with a product of the title compound having the anti-isomer configuration and not the syn-isomer as disclosed by said Czechoslovakian patent.

#### Example 13

##### Preparation of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

The experimental procedure of Example 2 in Czechoslovakian Patent No. 238,950 was repeated as follows:

A sample of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (4.0 g) having a KF of 0.06% was slurried in 20 ml of acetonitrile which had been adjusted to have a KF of 0.22%. A drop of dimethylformamide was added, and the temperature was 20 °C. On addition of 6.0 grams of powdered phosphorus pentachloride, the temperature rose to 40 °C and complete solution was obtained. The solution was cooled to 20 °C, and a precipitate formed at 33 °C. After stirring for 30 minutes, the product was collected under a dry nitrogen atmosphere and washed with 30 ml of benzene and 20 ml of heptane. Yield was 1.86 g after drying for 18 hours at 20 °-25 °C in vacuo over P<sub>2</sub>O<sub>5</sub>.

The product of the foregoing experiment was characterized by proton nuclear magnetic resonance spectrum in acetic acid-d<sub>4</sub> (<sup>1</sup>H NMR) as shown in Figure 3 which shows the H5 at 7.56 ppm and CH<sub>3</sub> at 4.31 ppm. The <sup>1</sup>H NMR spectrum is consistent with a product of the title compound having the anti-isomer configuration and not the syn-isomer as disclosed by said Czechoslovakian patent.

#### Example 14

##### Preparation of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

The experimental procedure of Example 3 in Czechoslovakian Patent No. 238,950 was repeated as follows:

Concentrated hydrochloric acid (0.16 ml) was added to 30 ml of dichloromethane. After cooling to -10 °C, 6.5 g of phosphorus pentachloride was added in increments. After warming to 0 °C, 4.0 g of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid having a KF of 0.06% was added in one aliquot. The temperature rose to 2 °C. A complete solution was obtained after 9 minutes at 0 °C. After 40 minutes, a precipitate started to form. The product slurry was agitated for 2.8 hours at 2 °-3 °C, filtered under a dry nitrogen atmosphere, washed with 30 ml of benzene and 20 ml of heptane, and dried in vacuo at 20 °-25 °C over P<sub>2</sub>O<sub>5</sub> for 18 hours. The yield was 3.42 g of light-yellow powder.

The product of the foregoing experiment was characterized by proton nuclear magnetic resonance spectrum in acetic acid-d<sub>4</sub> (<sup>1</sup>H NMR) as shown in Figure 4 which shows the H5 at 7.56 ppm and CH<sub>3</sub> at 4.31 ppm. The <sup>1</sup>H NMR spectrum is consistent with a product of the title compound having the anti-isomer configuration and not the syn-isomer as disclosed by said Czechoslovakian patent.

#### Example 15

##### Attempted preparation of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

The general procedure described in Example 7 of U.S. Patent No. 4,203,899 for the conversion of protected aminothiazolacetic acid to the corresponding acid chloride was applied to the unprotected aminothiazolacetic acid described herein as follows:

A sample of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt (2.38 g, 0.01 mole) was suspended in 30.5 ml of benzene and cooled to 20 °C. Oxalyl chloride (2.09 ml, 0.024 mole) was added, followed by dimethylformamide (0.50 ml, 0.0065 mole). The temperature rose to 22 °C with vigorous

gas evolution. Within 20 minutes at 20 °C, the gassing had subsided and the slurry was agitated at 20 °C ± 2 °C for 2 hours. The slurry was concentrated in vacuo to remove the solvent, and the resulting yellow product was dried in vacuo over P<sub>2</sub>O<sub>5</sub> at 20 °-25 °C for 16 hours. Yield was 2.59 g.

The product of the foregoing experiment was characterized by proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectrum in acetic acid-d<sub>4</sub> which shows the H5 at 7.60 ppm and CH<sub>3</sub> at 4.37 ppm. The spectrum of the product is consistent with the title compound having the anti-isomer configuration.

#### Example 16

##### 10 Attempted preparation of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

The general procedure described in Example 59 of U.S. Patent No. 4,203,899 for the conversion of protected aminothiazolacetic acid to the corresponding acid chloride was applied to the unprotected aminothiazolacetic acid described herein as follows:

15 A sample of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt (2.38 g, 0.01 mole) was suspended in 25 ml of dichloromethane. After cooling to 4 °C, 2.08 g (0.01 mole) of phosphorous pentachloride was added. With ice cooling, the temperature rose to 6 °C, and after cooling back to 4 °C, the slurry was stirred for 1 hour. The precipitate was collected by filtration under a dry nitrogen atmosphere, washed with dichloromethane (10 ml), and dried in vacuo at 20 °-25 °C to give 1.4 g of pale-yellow solid.

20 The product of the foregoing experiment was characterized by proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectrum in acetic acid-d<sub>4</sub> which shows the H5 at 7.61 ppm and the CH<sub>3</sub> at 4.34 ppm. The spectrum of the product is consistent with the title compound having the anti-isomer configuration. Additionally, the product is contaminated with unconverted acid (<sup>1</sup>H NMR having H5 at 7.07 ppm and CH<sub>3</sub> at 4.06 ppm) which was further confirmed by spiking with starting acid.

#### 25 Example 17

Acylation of 7-amino-3-[(1-methyl-1-pyrrolidinio)-methyl]-ceph-3-em-4-carboxylate HI salt using 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (anti-isomer from Example 14)

30 To a precooled solution of 9 ml acetone and 3.4 ml water at 10 °C was added 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt (1.13 g, 2.66 mmol). 2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetyl chloride hydrochloride (1.09 g, 4.21 mmol) [prepared in Example 14] was added in 5 portions at 0 °C along with triethylamine (0.37 ml, 2.66 mmol) to keep the pH at 6.0-7.0. The reaction mixture was stirred at ambient temperature for 15 minutes. Assay of the resulting solution by high pressure liquid chromatography (gradient C<sub>18</sub> column, 2% to 25% acetonitrile in 0.005M NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>) showed 72.4 area percent of anti-cefepime at 13.08 minutes and no detectable syn-cefepime which was expected at about 8.5 minutes retention time. Acidification with sulfuric acid to pH 1.9 gave 1.48 g of anti-cefepime as the sulfate salt. The identification of the product was confirmed by <sup>1</sup>H-NMR spectroscopy (DMSO-d<sub>6</sub>) and was shown to contain 0.58 mole of triethylamine salt.

#### Example 18

Acylation of 7-amino-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate HI salt using 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (anti-isomer from Example 14)

45 To a precooled solution of 108 ml of acetone and 40.5 ml of water at 10 °C was added 7-amino- [(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt (13.5 g, 0.0317 mol). The pH of the slurry was adjusted to 7.0 using 2.7 ml of 14% NH<sub>4</sub>OH. With the temperature at 10 °C, 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (13.05 g, 0.015 mol) [prepared by the procedure of Example 14] was added portionwise over 60 minutes while using 14% NH<sub>4</sub>OH (27 ml) to maintain a pH of 6.3-7.0 during the first half of the addition and at pH 6.1-6.6 during the second half of the addition. The reaction mixture was stirred at ambient temperature for 30 minutes. The reaction mixture was polished filtered and washed with 6 ml of 2:1 acetone/water then 6N H<sub>2</sub>SO<sub>4</sub> (15 ml) was slowly added to the filtrate to adjust the pH to 1.87-1.90. After stirring for 1 hour, the insolubles were filtered off and the cake washed with 21 ml of 2:1 acetone/water and then with 30 ml of acetone. To the filtrate was added 1 liter of acetone over 30 minutes and the mixture was stirred at 5-8 °C for 40 minutes. The product was collected by filtration, washed twice with 24 ml of 4:1 acetone/water, 60 ml of acetone and dried under vacuum to yield 20.64 g

(116% stoich weight) of anti-cefepime as the sulfate salt (95.4% pure by HPLC). The <sup>1</sup>H-NMR spectra was consistent with the structure of anti-cefepime containing about 3 moles of ammonium salts.

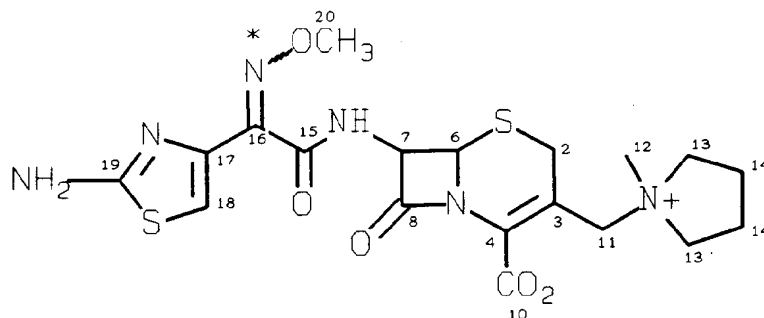
#### Example 19

Comparison of a product of Example 9 (cefepime syn-isomer) and a product of Example 17 (cefepime anti-isomer) show the following differences in physical characteristics.

High pressure liquid chromatography of the cefepime isomers was carried out on a Waters  $\mu$ Bondapak C<sub>18</sub> column (3.9 x 300 mm) using a solvent system mixture of 1000 ml water containing 2.88 g (0.013 mol) heptane sulfonic acid sodium salt and pH adjusted to 4.0 with acetic acid and 100 ml acetonitrile at a flow rate of 2.0 ml/minute. The products were visualized with a Water Model 450 variable wavelength detector set at 254  $\lambda$  to give the following results.

	Retention Time (min.)
Syn-isomer of cefepime (Example 9)	10.5
<u>Anti</u> -isomer of cefepime (Example 17)	37.8

The proton NMR spectra of the syn and anti-methoxime isomers of cefepime as the dihydrochloride salts was carried out on a Bruker AMX-400 FT NMR spectrometer using deuterated dimethyl sulfoxide as the solvent. The reported chemical shifts are referenced to DMSO at 2.49 ppm. The numbering system shown below in the Formula and Table are for convenience only.

Syn- and anti-cefepime

\* Wavy bond denotes syn- and anti-methoxyimino isomers.

Comparison Table of Proton NMR Chemical Shifts (ppm)		
Assignment	Syn-Cefepime	Anti-Cefepime
C2-H2	4.04, 3.65	4.02, 3.65
C6-H	5.33	5.31
C7-H	5.88	5.85
C11-H2	4.60, 4.31	4.59, 4.30
C12-H3	2.93	2.93
C13-H4	3.7, 3.4	3.6, 3.3
C14-H4	2.10	2.10
C18-H4	6.88	7.57
C20-H3	3.92	4.05
NH	9.83	9.56
NH2	8.60	8.70

The  $^1\text{H}$  NMR spectra of the two methoxime isomers of cefepime, as shown above, are significantly different. The thiazole ring CH(18) of the syn-(Z) methoxime isomer, at 6.88 ppm, falls upfield of the anti-(E) methoxime isomer CH(18), at 7.57.

Example 20

Anhydrous acylation of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt using 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (syn-isomer)

Under a nitrogen atmosphere, 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt (50 g, 0.1176 mol) was cooled to  $-20^\circ\text{C}$  in 500 ml of acetonitrile. Trimethylchlorosilane (39 ml, 2.5 eq.) and triethylamine (38 ml, 2.3 eq.) were added, keeping the temperature at or below  $-10^\circ\text{C}$ . After the silyl mixture was stirred for 1.5 hours at  $-10^\circ\text{C}$ , 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (syn-isomer obtained from Example 5) was added in two aliquots (15 g, 0.50 eq each). Another 8 ml (0.5 eq.) of triethylamine was added with 7.5 g (0.25 eq.) of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (syn-isomer). The slurry was stirred at  $-10^\circ\text{C}$  for 15 minutes, and then, 150 ml of water was added and stirred at ambient temperature to dissolve all solids. The acetonitrile layer was separated from the rich aqueous, and 6N HCl (2.5 eq.) was added to the aqueous with 400 ml of acetone. The solution was seeded and allowed to crystallize for 15 minutes. A further 1000 ml of acetone was added to complete crystallization. The slurry was stirred 1 hour and then filtered, washed with 400 ml of acetone, and dried at about  $40^\circ\text{C}$ . Yield was 56.51 g (84.1% stoich. weight) of cefepime (98.6% pure by HPLC assay as cefepime 2HCl  $\text{H}_2\text{O}$ ), which is identical to the cefepime 2HCl  $\text{H}_2\text{O}$  described by Kaplan, et al., in

U.S. Patent No. 4,910,301, issued March 20, 1990.

#### Example 21

##### 5    Anhydrous acylation of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt using 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (syn-isomer)

Under a nitrogen atmosphere, 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt (5.0g, 0.01176 mol) was cooled to 0-5 °C in 50 ml of acetonitrile. Trimethylchlorosilane (3.3 ml, 2.2 eq.) and N-methylmorpholine (2.7 ml, 2.1 eq.) were added, keeping the temperature  $\leq$  5 °C. The silyl mixture was stirred for 1.5 hours at 0-5 °C. 2-(2-Aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (1.5 g, 0.5 eq each) was added in two aliquots then stirred for 10 min. Another two aliquots of 2-(aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (1.5g, 0.5 eq each) with triethylamine (0.8 ml, 0.5 eq each) were added. The slurry was stirred at 0-5 °C for 1 hour then 15 ml of water was added and stirred at ambient temperature to dissolve all solids. The organic layer was separated from the rich aqueous, 6N HCl (5 ml, 2.5 eq.) and 60 ml acetone were added to the aqueous and stirred 15 minutes to begin crystallization. Another 80 ml of acetone was added to complete crystallization. The slurry was stirred 1 hour and then filtered, washed with 50 ml acetone, and dried at about 40 °C. Yield was 5.78 g (86.0% stoich. weight) of cefepime dihydrochloride monohydrate.

#### Example 22

##### 25    Anhydrous acylation of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt using 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (syn-isomer)

Under a nitrogen atmosphere, 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt (5.0 g, 0.01176 mol) was cooled to 0-5 °C in 50 ml of acetonitrile. Trimethylchlorosilane (1.34 ml, 0.90 eq.) and hexamethyldisilazane (1.8 ml, 0.75 eq.) were added keeping the temperature  $\leq$  5 °C. The silyl mixture was stirred for 1 hour at 0-5 °C. 2-(2-Aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (1.8 g, 0.59 eq. each) was added in two aliquots then stirred for 10 minutes. Another two aliquots of 2-(aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (1.5 g, 0.5 eq. each) with triethylamine (0.8 ml, 0.5 eq each) were added. The slurry was stirred at 0-5 °C for 1.5 hours then 15 ml water was added and stirred at ambient temperature to dissolve all solids. The organic layer was separated from the rich aqueous, 6N HCl (5 ml, 2.5 eq.) and 30 ml of acetone were added to the aqueous and stirred 10 minutes to crystallize the product. Another 110 ml of acetone was added to complete crystallization. The slurry was stirred for 1 hour and then filtered, washed with 75 ml of acetone, and dried at about 40 °C. Yield was 5.45 g (81.1% stoich. weight) of cefepime dihydrochloride monohydrate.

#### Example 23

##### 40    Anhydrous acylation of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt using 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (anti-isomer from Example 12)

Under a nitrogen atmosphere, 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt (2 g, 4.70 mmol) was cooled to -20 °C in 20 ml of acetonitrile. Trimethylchlorosilane (1.26 ml, 2.1 eq.) and triethylamine (1.32 ml, 2.05 eq.) were added keeping the temperature at or below -10 °C. After the silyl mixture was stirred for 1.5 hours at -10 °C, 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (anti-isomer obtained from Example 12) (1.2 g, 1.49 eq.) was added, and the reaction mixture was stirred for 1 hour at -10 °C.

The product of the above acylation mixture was compared to a similar anhydrous acylation mixture using syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride as in Example 20. The main product peak in the HPLC of the above acylation reaction did not correspond to the desired cefepime antibiotic. A sample of the product from the above acylation reaction was isolated by chromatography, and the <sup>1</sup>H NMR and HPLC data thereof are consistent with the anti-isomer of cefepime as described in Example 19.

## Example 24

Anhydrous acylation of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt using 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (syn-isomer)

5

Under a nitrogen atmosphere, 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt (10.0 g, 0.02252 mol) was stirred with 100 ml of dichloromethane at 20 °C. Trimethylchlorosilane (2.35 ml, 0.82 eq.) and hexamethyldisilazane (3.85 ml, 1.62 eq.) were added and the temperature of the silyl mixture was raised to 25 °C and held for 1.5 hours at 25 °C to 30 °C. The silyl mixture was then cooled to -40 °C and 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (6.04 g, 0.93 eq.) was added over 40 minutes at -40 °C to -20 °C. Triethylamine (1.65 ml, 0.5 eq.) and 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (1.21 g, 0.19 eq.) were added and the slurry held at -20 °C to 25 °C for 65 minutes. The slurry was then added to water (50 ml) over 10 minutes and stirred at ambient temperature for 1 hour to dissolve most of the solids. Celite (0.5 g) was added, the mixture filtered and the cake washed with dichloromethane (10 ml). The organic layer was separated from the rich aqueous layer and concentrated sulfuric acid (5.9 ml, 0.111 mol) was added to the rich aqueous over 5 minutes at 20 °C to 25 °C. Acetone (320 ml) was then added to the rich aqueous over 35 minutes to crystallize the product. The slurry was held 20 minutes at ambient temperature and was then cooled to 0-5 °C for 1 hour. The slurry was filtered, washed with acetone (150 ml) and the solid dried at about 40 °C. The yield was 11.54 g (84.9% stoich. weight) of cefepime sulfate.

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## Example 25

Anhydrous acylation of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt using 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (syn-isomer)

25

Under a nitrogen atmosphere, 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt (10.0 g, 0.02252 mol) was stirred with 100 ml of dichloromethane at 20 °C. Trimethylchlorosilane (2.35 ml, 0.82 eq.) and hexamethyldisilazane (3.85 ml, 1.62 eq.) were added and the temperature of the silyl mixture was raised to 25 °C and held for 1.5 hours at 25 °C to 30 °C. The silyl mixture was cooled to -40 °C and 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (6.04 g, 0.93 eq.) was added over 40 minutes at -40 °C to -20 °C. Triethylamine (1.65 ml, 0.5 eq.) and 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (1.21 g, 0.19 eq.) were added and the slurry held at -20 °C to 25 °C for 45 minutes. The slurry was added to water (45 ml) over 10 minutes and stirred at ambient temperature for 1 hour to dissolve most of the solids. Celite (0.5 g) was added and the mixture filtered through a Celite (1.0 g) pad. The organic layer was separated from the rich aqueous. The rich aqueous was twice stirred with carbon (1.0 g each) and filtered. The combined carbon cake was washed with a solution of water (10.5 ml), 12N HCl (5 ml, 2.5 eq.) and acetone (20.5 ml). Acetone (320 ml) was then added to the combined filtrate and wash over 35 minutes to crystallize the product. The slurry was held 30 minutes at ambient temperature and was then cooled to 0 °C for 1 hour. The slurry was filtered, washed with 80 ml of acetone and the solid dried at about 40 °C. The yield was 10.25 g (76.3% stoich. weight) of cefepime dihydrochloride monohydrate.

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## Example 26

Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt

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Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (85.3 g, 424 mmol) in dichloromethane (570 ml) was milled under nitrogen for 15 minutes in a blender. The resulting fine suspension was diluted with dichloromethane (100 ml), and transferred under nitrogen to a 1L Buchi jacketed reactor. The reactor was pressurized with nitrogen (5 psi) and the mixture stirred at 375 rpm and cooled to -2 °C. Hydrogen chloride (15.4 g, 424 mmol) was introduced into the headspace of the reactor at 0.2 g per minute. There was a temperature rise of 2 °C. The mixture was stirred for a further 30 minutes at 0 °C, filtered, and washed with dichloromethane (350 ml) under nitrogen. The solid was dried in vacuo at 45 °C for 18 hours. The title compound was an off-white powder (110.9 g, 111% uncorrected yield).

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Anal. Calcd. for C <sub>6</sub> H <sub>8</sub> N <sub>3</sub> O <sub>3</sub> SCl:			
	C, 30.32; S, 13.49;	H, 3.39; Cl, 14.91;	N, 17.68;
Found:	C, 29.37; S, 12.70;	H, 3.17; Cl, 16.99.	N, 16.34;

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 4.05 (s, 3H, CH<sub>3</sub>), 5.9 (s, 15 mole % residual CH<sub>2</sub>Cl<sub>2</sub>), 7.1 (s, 1H, C-5 H). Signals were also seen at 4.18 (s, 3H, CH<sub>3</sub>) and 7.7 (s, 1H, C-5 H) corresponding to ca. 2% of anti-isomer.

#### Example 27

##### Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt

Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (25 g, 124 mmol) in acetonitrile (125 ml) under nitrogen was titrated with a 1.39M solution of HCl in acetonitrile (89.2 ml, 123.9 mmol) and was maintained at 10° to 15° C. The mixture was stirred for a further 30 minutes at 10° to 15° C, filtered, and washed with acetonitrile (200 ml) under nitrogen. The solid was dried in vacuo at 45° C for 3 hours. The title compound was an off-white powder (29.5 g, 97.4% uncorrected yield).

<sup>1</sup>H NMR (CD<sub>3</sub>OD) δ: 2.05 (s, 13% wt/wt, residual acetonitrile), 4.1 (s, 3H, CH<sub>3</sub>), 7.1 (s, 1H, C-5 H). Signals were also seen at 4.2 (s, 3H, CH<sub>3</sub>) and 7.8 (s, 1H, C-5 H) corresponding to ca. 0.5% of anti-isomer.

#### Example 28

##### Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride (56.24 g, 210 mmol), containing approximately 11% wt/wt residual acetonitrile) in dichloromethane (450 ml) was milled under nitrogen for 3 minutes in a blender, then cooled to -35° C and transferred under nitrogen over 5 minutes to a well stirred slurry of Vilsmeier reagent, also at -35° C. The slurry of Vilsmeier reagent was prepared by adding oxalyl chloride (28.2 g, 221 mmol) portionwise to a solution of dimethylformamide (16.89 g, 231 mmol) in dichloromethane (300 ml) at 0° C, followed by cooling to -35° C. During the addition, the reaction temperature rose to -28° C. After the addition, the reaction mixture was seeded with the product. After a further 2.5 hours at -28° C to -35° C the mixture was filtered and the filter cake washed with dichloromethane (200 ml) under nitrogen. Nitrogen was passed through the cake for 30 minutes, then the solid was dried in vacuo at room temperature for 12 hours. The title compound was obtained as an off-white powder (42.9 g, 72% yield).

<sup>1</sup>H NMR (CD<sub>3</sub>OD) δ: 4.06 (s, 3H, CH<sub>3</sub>), 7.12 (s, 1H, C-5 H). Signals were seen at 7.18 corresponding to ca. 5% of the acid hydrochloride salt, and at 7.80 (s, C-5 H) corresponding to ca. 0.5% of the anti-isomer. After derivatization with diethylamine in acetonitrile, HPLC analysis showed the title compound (syn-isomer, as its diethylamide derivative) with a retention time of 9.6 minutes, the acid hydrochloride salt with a retention time of 2.8 minutes, and the anti-isomer (as its diethylamine derivative) with a retention time of 16.4 minutes. The ratio of the syn-isomer:acid hydrochloride salt:anti-isomer was 90:5:<1.

#### Example 29

##### Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (84.7 g, 421 mmol) in dichloromethane (570 ml) was milled under nitrogen for 20 minutes in a blender. The resulting fine suspension was diluted with dichloromethane (100 ml), and transferred under nitrogen to a 1L Buchi jacketed reactor. The reactor was pressurized with nitrogen (5 psi) and the mixture stirred at 375 rpm and cooled to -2° C. Hydrogen chloride (15.3 g, 421 mmol) was introduced into the headspace of the reactor at 0.2 g per minute. There was a temperature rise of 2° C. The mixture was stirred for a further 30 minutes at 0° C, milled for 3 minutes in the blender, then cooled to -35° C and transferred under nitrogen over 5 minutes to a well stirred slurry of Vilsmeier reagent, also at -35° C. The slurry of Vilsmeier reagent was prepared by adding oxalyl chloride (56.1 g, 439 mmol) portionwise to a solution of dimethylformamide (33.8 g, 462 mmol) in dichloromethane

(880 ml) at 0 ° C, followed by cooling to -35 ° C. During the addition, the reaction temperature rose to -28 ° C. After the addition, the reaction mixture was seeded with product. After a further 2.5 hours at -28 ° C to -35 ° C the mixture was filtered and the filter cake washed with dichloromethane (350 ml) under nitrogen. Nitrogen was passed through the cake for 30 minutes, then the solid was dried *in vacuo* at room temperature for 12 hours. The title compound was obtained as an off-white powder (95.2 g, 89% uncorrected yield).

Anal. Calcd. for C <sub>6</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub> SCl <sub>2</sub> :			
	C, 28.14; S, 12.52;	H, 2.76; Cl, 27.68;	N, 16.41;
Found:	C, 28.11; S, 12.22;	H, 2.62; Cl, 26.74.	N, 16.20;

<sup>1</sup>H NMR (CD<sub>3</sub>OD) δ: 4.06 (s, 3H, CH<sub>3</sub>), 7.12 (s, 1H, C-5 H). Signals were also seen at 7.18 (s, C-5 H) corresponding to ca. 4% of acid hydrochloride salt, and at 7.80 (s, C-5 H) corresponding to ca. 2% of the anti-isomer. After derivatization with diethylamine in acetonitrile, HPLC analysis showed the title compound (as its diethylamide derivative) with a retention time of 9.6 minutes, the starting acid with a retention time of 2.8 minutes, and the anti-isomer (as its diethylamide derivative) with a retention time of 16.4 minutes. The ratio of syn-isomer:starting acid:anti-isomer was 90:4:2.

#### EXAMPLE 30

##### Conversion of Cefepime Dihydrochloride Monohydrate to Cefepime Dihydrochloride Dihydrate

Cefepime dihydrochloride monohydrate (300 g, purity by HPLC 99.9%, KF 3.8%) was dissolved in deionized water (1200 ml). Hydrochloric acid 6N (132 ml, 1.5 equivalent) was added. The solution was filtered and washed with deionized water (300 ml).

Acetone (1500 ml) was added to the filtered solution. Additional acetone (4000 ml) was added dropwise over 20 minutes. The solution was held at cloud point until heavy dihydrate crystals formed (needles by microscopic analysis; seeding optional at cloud point). Additional acetone (8000 ml) was added over a 25 minute period. The thick slurry was stirred at 25 ° C for one hour.

The crystal form was confirmed to be dihydrate (needles) by microscopic analysis comparing the crystals to an authentic sample. The slurry was filtered and washed with acetone (2 x 1500 ml). The cake was dried at 40 ° C *in vacuo* for 15 hours. The yield of cefepime dihydrochloride dihydrate was 305.10 g (98.6%), purity by HPLC 99.0%, KF 6.5%.

#### EXAMPLE 31

##### Conversion of Cefepime Dihydrochloride Dihydrate to Cefepime Dihydrochloride Monohydrate

Cefepime dihydrochloride dihydrate (15.0 g, purity by HPLC 99.2%, KF 6.4%) was dissolved in deionized water (75 ml). Hydrochloric acid 6N (0.9 ml, 0.2 equivalent) was added. The solution was filtered through a .45 micron filter.

Acetone (200 ml) was added dropwise to the filtered solution over 20 minutes to give a cloudy solution (optional seeding at this point). Without holding at this point more acetone (400 ml) was added dropwise over 40 minutes. The slurry was cooled in a ice bath at 0 ° to 5 ° C for one hour.

The crystal form was confirmed to be monohydrate by microscopic analysis comparing the crystals to an authentic sample. The slurry was filtered and washed with acetone (2 x 60 ml). The cake was dried at 40 ° C *in vacuo* for 15 hours. The yield of cefepime dihydrochloride monohydrate was 13.28 g (91.8%), and the crystal structure was confirmed to be the same as described by Kaplan, et al in U.S. Patent No. 4,910,301.

## Example 32

## Preparation of 7-[2-[2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-methyl]-ceph-3-em-4-carboxylate dihydrochloride hydrates

Under an inert atmosphere at room temperature, 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate hydriodide (14.67 g; 0.0345 mol) was suspended in dichloromethane (150 ml). Trimethylchlorosilane (4.7 ml) and hexamethyldisilazane (7.7 ml) were added to the slurry and the mixture was warmed to 25 - 30 °C over 1.5 hours. The reaction was then cooled to about -50 °C, and syn-2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (7.24 g; 0.0283 mol) was added in three portions over 35 minutes while the temperature gradually rose to -30 °C. Triethylamine (1.47 ml) and 1.78 g (0.0069 mol) of syn-2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride were added and the acylation continued between -20 °C and -25 °C for approximately 1 hour. Once the reaction was complete (as indicated by HPLC), the mixture was warmed to -5 °C and then 56 ml of water and 10 ml of dimethylacetamide were added. The reaction slurry was stirred at 25 °C until the solids dissolved. The phases were separated and the aqueous phase was polish filtered. The aqueous phase was decolorized with activated carbon (3 g), filtered and the filtrate was divided into two equal portions.

## Method A

One portion of the rich aqueous filtrate was acidified with 12N hydrochloric acid (11.7 ml; 0.14 mol). The slurry was seeded with cefepime dihydrochloride dihydrate (0.5 g) and the slurry was heated at 40 °C for 1 hour then held at room temperature overnight. The mixture was diluted further with acetone (126 ml), stirred at room temperature for 0.5 hour, then cooled at 0 - 5 °C for 1 hour. The product was collected by filtration, washed with acetone and dried in vacuo at 45 °C. The dihydrochloride dihydrate form of the title compound (needle-like crystals) was obtained with a 96.3% purity (9.11 g; 87.6% stoichiometric weight yield). Water content by Karl Fischer analysis was 6.3% and FT-IR (Diffuse Reflectance with KBr) analysis showed absorbance peaks at 3574 cm<sup>-1</sup> and 3432 cm<sup>-1</sup>.

## Method B

The second portion of rich aqueous filtrate was acidified with 6N hydrochloric acid (15 ml; 0.09 mol), diluted with acetone (280 ml) over 20 minutes then cooled at 0 - 5 °C for 1 hour. The slurry was filtered, washed with acetone and dried in vacuo at 45 °C. The dihydrochloride monohydrate form (granular-like crystals) of the title compound was obtained with 95.5% purity (8.38 g; 83.1% stoichiometric wt. yield). Water content by Karl Fischer analysis was 3.9%.

## Claims

1. A process for the preparation of antibiotic, cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the  $\Delta^2$  isomer which comprises reacting a silylated derivative of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate with the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer in an inert organic solvent.
2. A process of Claim 1 further comprising the preparation of said syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer by reacting the anhydrous acid hydrochloride salt of the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid with a mixture containing at least one molar equivalent of oxalyl chloride and at least one molar equivalent to a slight excess of dimethylformamide to an amount of said oxalyl chloride in an inert organic solvent at a temperature of less than -10 °C.
3. A process of Claim 1 further comprising the preparation of said silylated derivative by reacting 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate salt in an inert organic solvent with a silylating agent.
4. A process of Claim 1 further comprising the addition of less than one molar equivalent of a base.

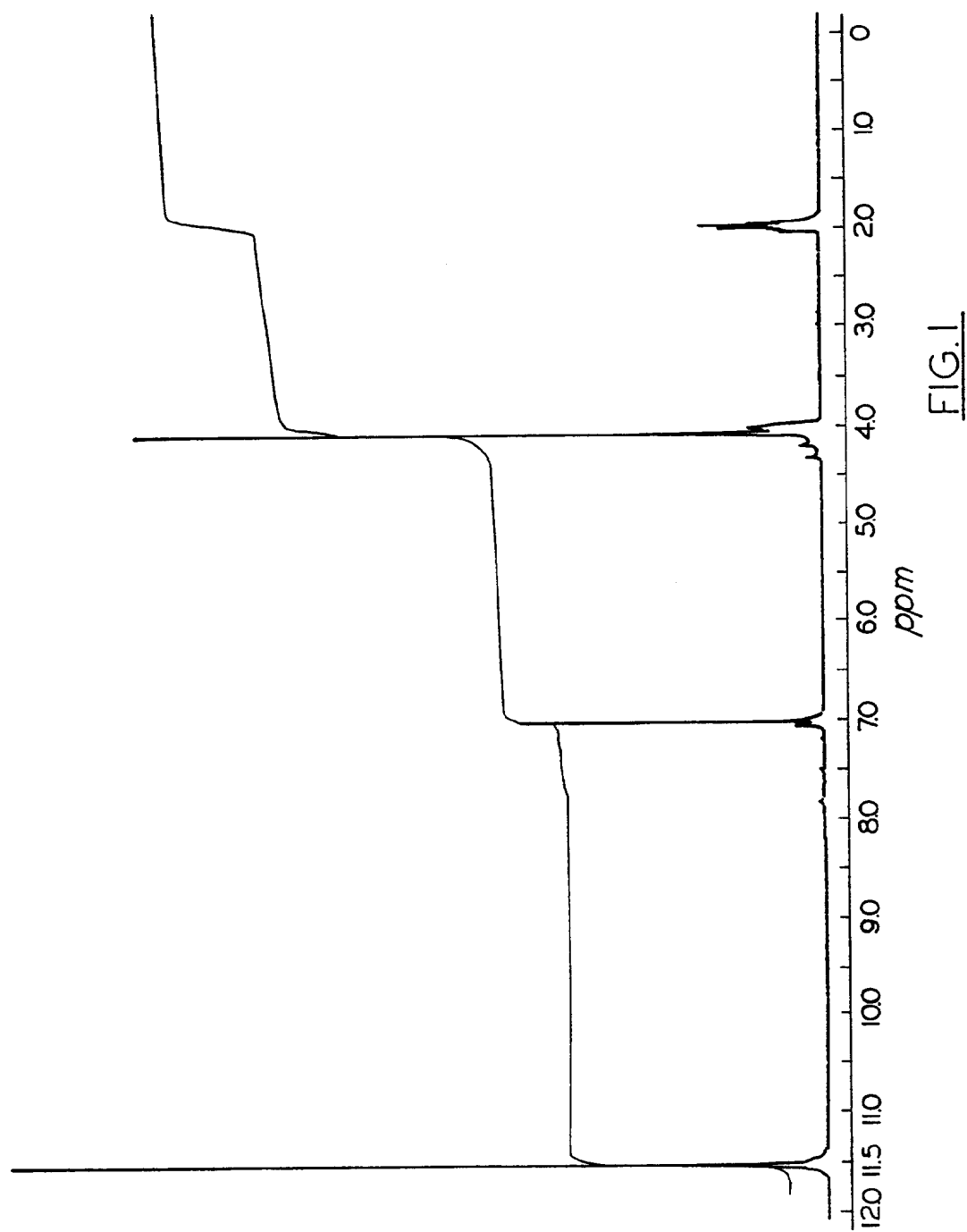
5. A process of Claim 1 further comprising the addition of a sufficient amount of water to the reaction mixture to produce an organic-aqueous diphasic solution; and then adding a sufficient amount of an acid or soluble non-toxic salt thereof to the separated aqueous solution.
- 5 6. A process of Claim 5 wherein the acid is hydrochloric acid to produce said antibiotic.
7. A process of Claim 5 wherein the acid is sulfuric acid.
8. A process of Claim 7 further comprising the neutralization of the resulting sulfate salt with base followed  
10 by the addition of a sufficient amount of hydrochloric acid to produce said antibiotic.
9. A process of Claim 8 wherein the base is a weakly basic ion-exchange resin.
10. A process of Claim 2 wherein the amount of oxalyl chloride is 1.0 to about 2.0 molar equivalents and  
15 the amount of dimethylformamide is a slight equimolar excess of said oxalyl chloride.
11. A process of Claim 10 wherein the amount of oxalyl chloride is 1.05 molar equivalents and the amount of dimethylformamide is 1.075 equivalents to said anhydrous acid hydrochloride salt.
- 20 12. A process of Claim 2 wherein the temperature is about -15 ° C to -40 ° C.
13. A process of Claim 3 wherein the silylating agent is a mixture of trimethylchlorosilane and hexamethyl-  
disilazane.
- 25 14. A process of Claim 4 wherein the base is N-methylmorpholine or triethylamine.
15. A process of Claim 13 wherein the amount of silylating agent is about 2.0 to about 2.5 molar  
equivalents.
- 30 16. A process of Claim 1, 2 or 3 wherein the inert organic solvent is acetonitrile or dichloromethane.
17. A process of Claim 1 wherein said antibiotic contains from 2.5% to 7.0% by weight of water.
18. A process of Claim 1 wherein said antibiotic is cefepime dihydrochloride monohydrate.
- 35 19. A process of Claim 1 wherein said antibiotic is cefepime dihydrochloride dihydrate.

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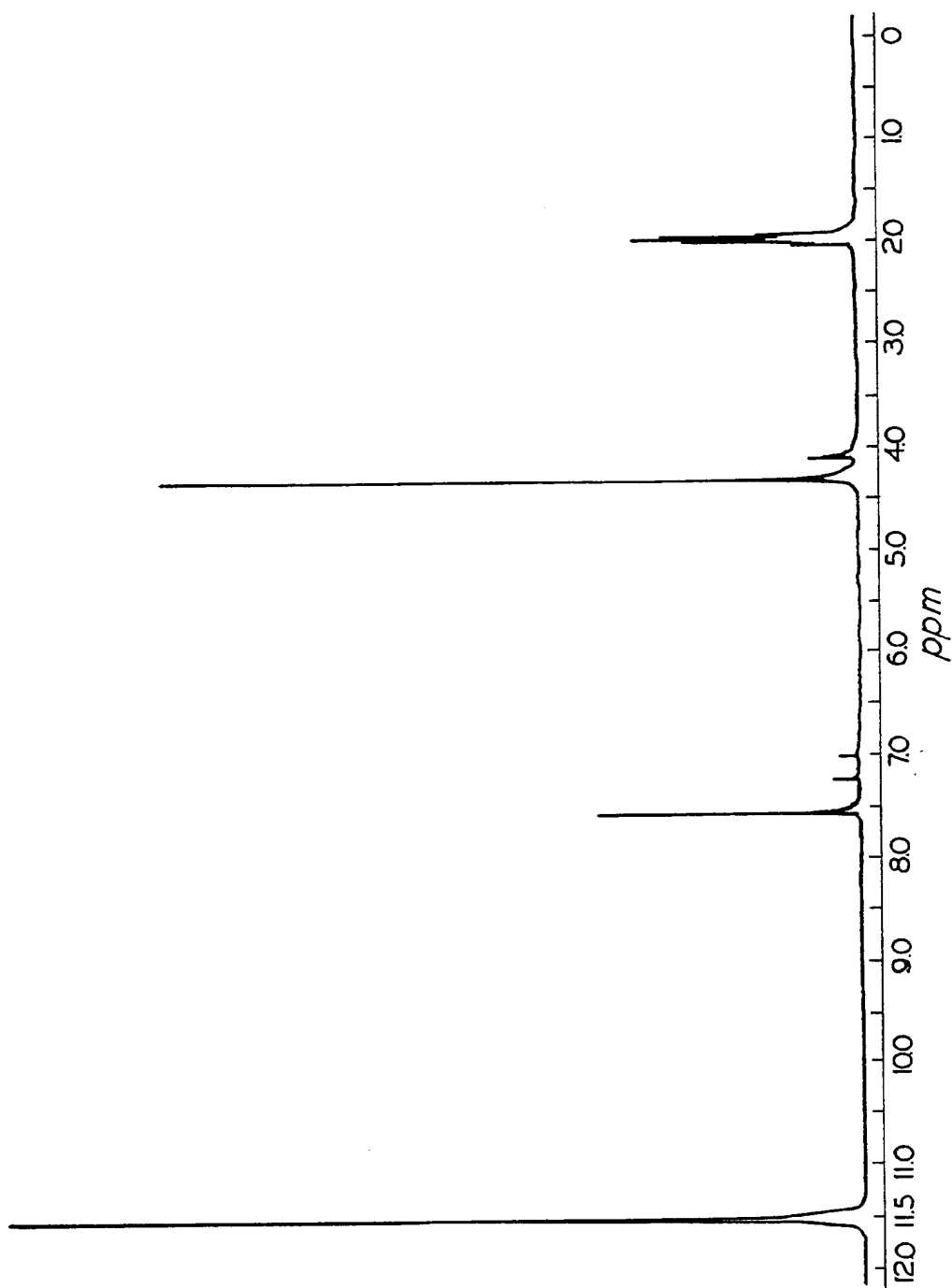


FIG. 2

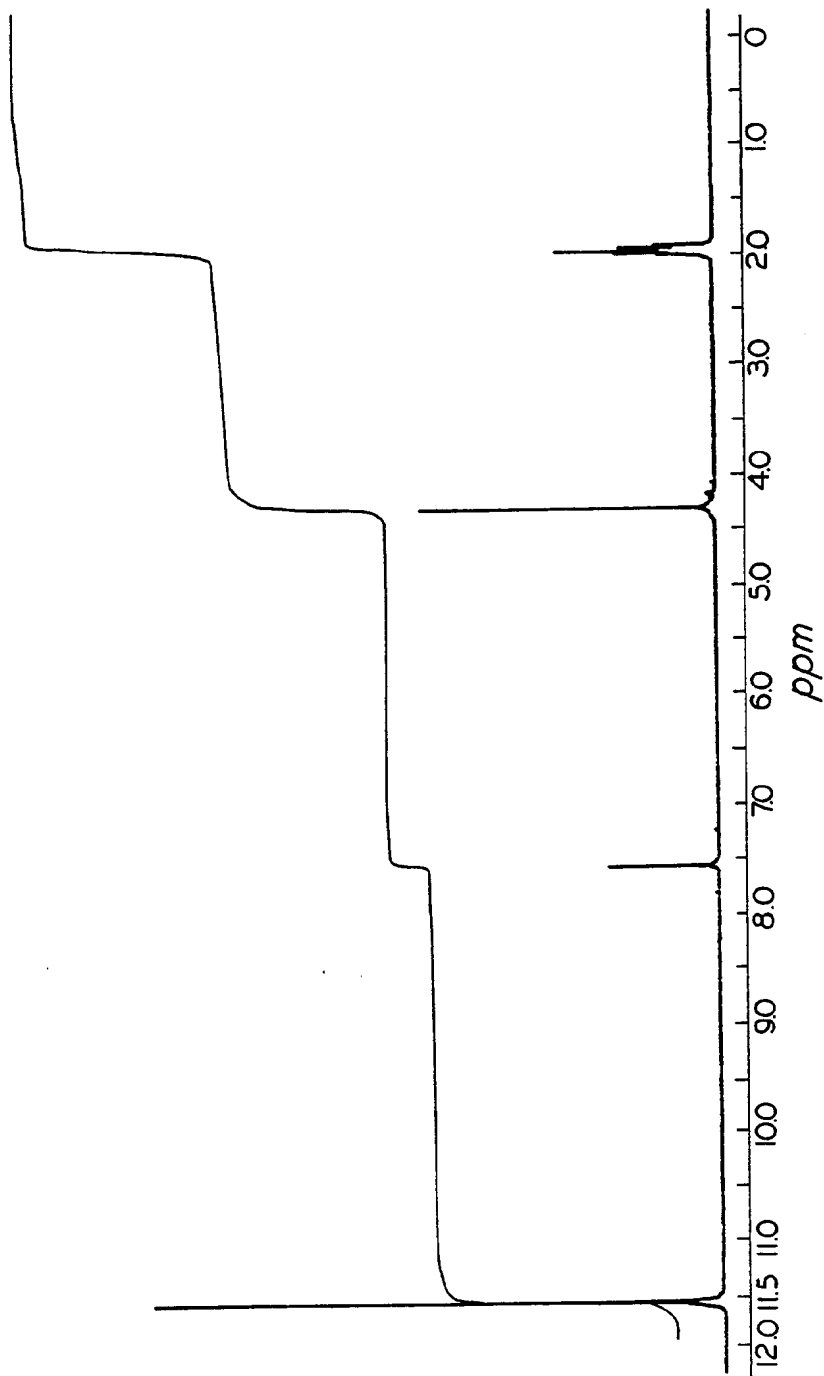


FIG. 3

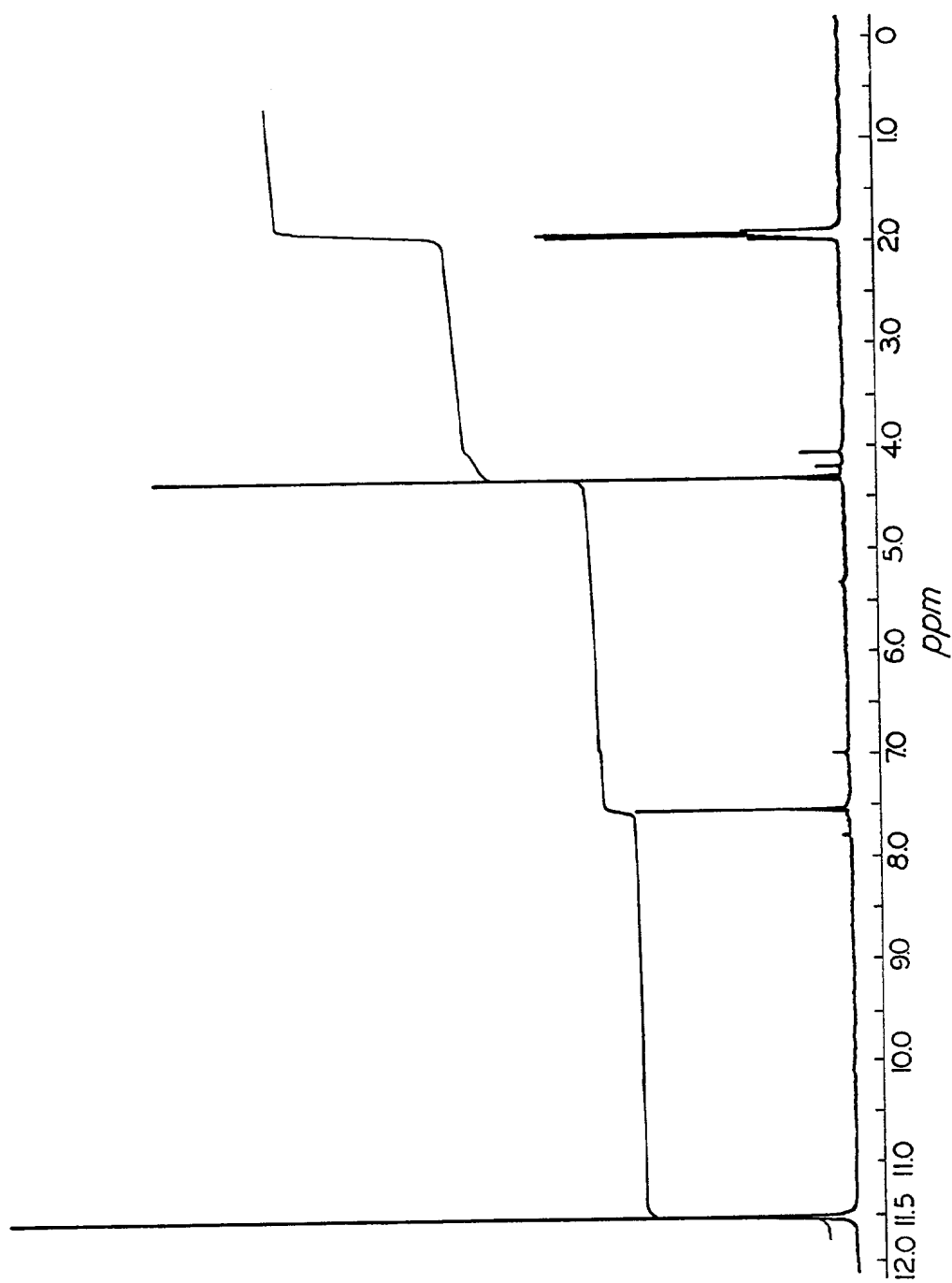


FIG. 4



European Patent  
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## EUROPEAN SEARCH REPORT

Application Number

EP 92 11 5443

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,X	CHEMICAL ABSTRACTS, vol. 110, no. 5, 30 January 1989, Columbus, Ohio, US; abstract no. 38979d, page 544 ;column L ; * abstract * & CS-A-238 950 (NOVAK LUDVIK ET. AL.) ---	1-2	C07D501/00 C07D277/20 C07F7/10 C07F7/18
A	CHEMICAL ABSTRACTS, vol. 113, no. 3, 16 July 1990, Columbus, Ohio, US; abstract no. 23533s, page 624 ;column R ; * abstract * & JP-A-0 200 790 (FUJISAWA) ---	1	
D,A	GB-A-2 165 245 (GLAXO) *Complete specification* & US-A-4 943 631 (BRIAN EDGAR LOOKER) ---	1	
D,A	EP-A-0 162 395 (BAYER) *Complete specification* & US-A-4 754 031 (ANGELBAUER ROLF) ---	1	
D,A	WO-A-8 701 116 (BRISTOL- MYERS) *Complete specification* & US-A-4 868 294 (BRUNDIDGE STEVEN ET. AL.) ---	1	TECHNICAL FIELDS SEARCHED (Int. Cl.5)
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D,A	GB-A-2 116 180 (BRISTOL MYERS) *Complete specification* & US-A-4 406 899 (SHIMPEI ABURAKI ET. AL.) -----	1	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 20 JANUARY 1993	Examiner LUYTEN H.W.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document	